IS THE SPECIFICITY OF THE ANAPHYLAXIS REACTION DEPENDENT ON THE CHEMICAL CONSTITUTION OF THE PROTEINS OR ON THEIR BIOLOGICAL RELATIONS?*

THE BIOLOGICAL REACTIONS OF THE VEGETABLE PROTEINS. II.

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In a previous paper[2] it was noted that zein, the alcohol-soluble protein of corn, did not cause the anaphylaxis reaction in animals sensitized with gliadin, the alcohol-soluble protein of wheat, nor with hordein, the alcohol-soluble protein of barley. At that time hordein and gliadin had not been tested against one another, but it had been observed that preparations of gliadin, from either wheat or rye, interacted against one another[3] as if they were one and the same protein. On testing hordein from barley against gliadin from either wheat or rye, we have since found that these two proteins of different origin also react well with one another. Furthermore, we have found that while our preparations of gliadin from wheat react anaphylactically with glutenin from the same seed, hordein from barley fails to cause reactions in guinea-pigs sensitized with glutenin. These results have raised the question whether the specificity of the anaphylaxis reaction is not, in fact, determined solely by the chemical constitution of the proteins used as antigens.

It is true that the anaphylaxis reaction has been obtained between fluids and extracts of tissues of animals or plants of different, though closely related, species, but these fluids, or extracts, have been composed of mixtures of so many substances of practically unknown chemical constitution that attention hereto-

* Received for publication April 10, 1913.

[1] A portion of the expenses of this investigation was also shared by the Carnegie Institution of Washington, D.C.

[2] Wells and Osborne, *Jour. Infect. Dis.*, 1911, 8, p. 66.

[3] For the avoidance of circumlocution the phrase "interacted with one another" is used, meaning that animals sensitized with one protein reacted when given the other, and animals sensitized with the second protein reacted when given the first. Throughout the article wherever it is stated that one protein does or does not react with another, it is to be understood that this is used as an abbreviation to indicate the above relationship.

fore has been directed almost exclusively to the biological relations of the substances causing the anaphylaxis reaction, and relatively little consideration has, as yet, been given to their possible chemical relations.

It is impossible to state how nearly related wheat and barley actually are, for the morphological characters, on which botanical classification is based, are not necessarily accurate indices of biological relationships. However closely akin wheat and barley may be, we have in our results an instance in which an isolated and purified protein constituent of the seed of one species reacts biologically with one of the isolated proteins of the seed of another species, but not with a second purified protein from the same seed, although the two proteins from the latter seed produce severe reactions when tested against one another.

We have, furthermore, found evidence of two reacting elements in gliadin and hordein, one of which is common to both proteins. Whether this means that our preparations of gliadin and hordein consist of two or more proteins, one of which is common to both the preparations which we designate gliadin and hordein, or that gliadin and hordein are single chemical individuals which contain two or more groups capable of acting as specific antigens, cannot be settled, for as yet we have no means by which the chemical individuality of any protein can be definitely established.

The physical and chemical properties of all proteins are such that none of the means employed for establishing the chemical individuality of organic compounds can be applied to them. The best that can be done at present is to subject the isolated products to extensive fractional precipitation and to determine whether, or not, differences can be detected between the various fractions thus obtained. Such examinations have been made of the proteins used in this investigation, and no evidence was secured which indicated that any of them were mixtures. The earlier view of Ritthausen that preparations of gliadin and hordein, as made by our methods, contain two or more different proteins was not established by the extensive fractionations made by one of us, and subsequent experience has further confirmed our view that each of these preparations contains only a single protein. Of course the character of the evidence thus obtained does not preclude the possibility that

these, like all other so-called individual proteins, are, in fact, mixtures of two or more different, but similar, proteins. Such an assumption, however, is purely speculative, and, as yet, has only a little indirect experimental evidence to support it.[1]

The main point of interest for us at the present time is that in the reactions described in this paper we have a definite indication that *the specificity of the anaphylaxis reaction is determined by the chemical structure of the reacting proteins rather than by their biological origin.*

While gliadin and hordein differ in their ultimate composition, and also in the proportion of some of the products which they yield on hydrolysis, to such an extent that a difference in constitution is plainly shown, nevertheless they resemble one another so closely in physical properties and amino-acid make-up that *similarity* in chemical constitution is not at all improbable.

Glutenin, on the other hand, differs so distinctly from gliadin and hordein, both in solubility and amino-acid make-up, that there can be no question that it represents quite a different type of protein.

One striking feature of the reactions between gliadin and glutenin is that when the sensitizing dose is glutenin and the intoxicating dose also glutenin the reaction is less severe than when the sensitizing dose is gliadin and the intoxicating dose is glutenin. From this fact it is almost certain that the reaction between these proteins is not caused by contamination of the glutenin with traces of gliadin. Whether or not our preparations of gliadin were contaminated with traces of glutenin cannot be determined with absolute certainty, but the methods of preparation, and the differences in solubility of these two proteins make it far less probable that the gliadin contains any glutenin, than that the glutenin contains a trace of gliadin.

The most reasonable explanation of these results is that both gliadin and glutenin contain common groups which react with one another, and that the specificity of the anaphylaxis reaction is not dependent on the chemical make-up of the *entire protein molecule.* This view is further supported by the fact that *while animals sensitized with glutenin react to an intoxicating dose of gliadin, they*

[1] Cf. Osborne, *The Harvey Lectures*, 1910–1911, p. 67; also Abderhalden and Fodor, *Ztschr. f. physiol. Chem.*, 1912, 81, p. 1.

do not react to an intoxicating dose of hordein. These facts are in harmony with the evidence of two reacting elements in gliadin, which is also further shown by our saturation experiments with gliadin and hordein. In accord with these observations, the assumption does not appear unjustified that gliadin contains one group which reacts with hordein, and another which does not react with hordein, but does react with glutenin, this latter group being absent from hordein. In this connection the anaphylaxis reaction with a 14-member polypeptide, 1-leucyl-triglycyl-1-leucyl-octaglycyl-glycin, recently reported by Abderhalden[1] is of interest as suggesting the possibility of separate reactive groups within a single protein molecule.

Although our results do not give us an insight into the precise manner in which the anaphylaxis reaction is brought about, they do furnish the most definite indication yet obtained that the specificity of this reaction is determined by the chemical constitution of the proteins which cause it.

EXPERIMENTAL PART.

The proteins used in this investigation were gliadin and glutenin from wheat, gliadin from rye, and hordein from barley. Gliadin and glutenin together, and in approximately equal quantity, constitute about 80 per cent of the proteins of the seed of wheat (*Triticum vulgare*), from which they can be removed in the form of the familiar wheat gluten by washing the ground seed with water. Of this gluten, gliadin and glutenin form the chief part. Gliadin can be extracted from the gluten, or directly from the ground seed, by 70 per cent alcohol, in which it is freely soluble, but in which glutenin does not dissolve.

Our preparation of gliadin was extracted from gluten by 70 per cent alcohol and purified by repeatedly pouring its clear and concentrated alcoholic solution alternately into large volumes of cold water and absolute alcohol, until all the non-gliadin substances soluble in either of these solvents were, as far as possible, removed. The snow-white, friable product finally obtained was then digested with absolute ether, until everything soluble therein was extracted.

[1] *Ztschr. f. physiol. Chem.*, 1912, 81, p. 322.

The glutenin was brought into solution by means of very dilute alkali, precipitated by neutralizing its perfectly clear alkalin solution with hydrochloric acid, and the precipitate extracted with 70 per cent alcohol in order to remove gliadin. By five reprecipitations and digestions with 70 per cent alcohol, the gliadin was nearly all extracted, and then by repeated and prolonged digestions with 70 per cent alcohol until only the merest traces of anything was dissolved, the remainder was practically completely removed. The glutenin was then digested with absolute alcohol and ether, and obtained as a fine, snow-white powder.

The rye gliadin and the hordein were obtained by directly extracting the flour of rye (*Secale cereale*), and the flour of barley (*Hordeum vulgare*), respectively with 70 per cent alcohol, and purified by the same process as that employed in preparing the gliadin from wheat.

The similarities and differences between these three proteins are shown in the following table:[1]

	Gliadin Wheat	Gliadin Rye	Glutenin Wheat	Hordein Barley
Solubility—				
Water	Slightly sol.	Slightly sol.	Insol.	Slightly sol.
Abs. alcohol	Insol.	Insol.	Insol.	Insol.
Dil. alcohol	Very sol.	Very sol.	Insol.	Very sol.
Dil. aqueous acids	Sol.	Sol.	Sol.	Sol.
Dil. aqueous alkalies	Sol.	Sol.	Sol.	Sol.
Ultimate composition—				
Carbon	52.72	52.75	52.34	54.29
Hydrogen	6.86	6.84	6.83	6.80
Nitrogen	17.66	17.72	17.49	17.21
Sulphur	1.02	1.21	1.08	0.83
Oxygen	21.74	21.48	22.26	20.87
	100.00	100.00	100.00	100.00
Products of hydrolysis—				
Glycocoll	0.00	....	0.89	0.00
Alanine	2.00	....	4.65	1.34
Valine	3.34	....	0.24	1.40
Leucine	6.62	....	5.95	7.00
Proline	13.22	....	4.23	13.73
Phenylalanine	2.25	2.70	1.97	5.48
Aspartic acid	0.58	....	0.91	1.32
Glutaminic acid	43.66	....	23.42	43.20
Serine	0.13	....	0.74	0.10
Tyrosine	1.20	1.19	4.25	1.67
Cystine	0.45	....	0.02	?
Lysine	0.00*	0.00	1.92	0.00
Histidine	1.63	....	1.76	1.28
Arginine	2.78	2.22	4.72	3.14
Tryptophane	1.00 (about)	Present	Present	Present
Ammonia	5.22	5.11	4.01	4.87
	84.08	.....	59.68	84.53

* It is possible that even the purest gliadin may contain a very small amount of lysine. Cf. Osborne and Mendel, *Jour. Biol. Chem.*, 1912, 12, p. 473.

[1] For an account of the properties of these proteins and a discussion of the literature relating to them, see Osborne, *Ergebnisse der Physiologie*, 1910, 10, p. 62.

The results obtained when guinea-pigs were injected with hordein and gliadin dissolved in 0.1 per cent NaOH are shown in Table 1.

TABLE 1.

INTERACTION OF HORDEIN AND GLIADIN.

Sensitizing Dose gm.	Second Dose 0.100 gm. (15–21 Days Later)	Result *	Third Dose 0.100 gm. (24–48 Hrs. Later)	Result
Hordein, barley—				
1. 0.010	Gliadin, wheat	Severe	Hordein, barley	Slight
2. 0.002	" "	"	" "	"
3. 0.001	" "	"	" "	"
4. 0.0002	" "	"	" "	Severe
5. 0.010	" "	Died, 25 min.	.....	.....
6. 0.002	" "	" 15 "	.....	.....
7. 0.001	" "	Severe	Hordein, barley	Severe
8. 0.0002	" "	"	" "	"
9. 0.010	" "	"	" "	"
10. 0.002	" "	Died, 18 min.	.....	.....
11. 0.001	" "	Severe	Hordein, barley	Severe
12. 0.0002				
Gliadin, wheat—				
13. 0.010	Hordein, barley	Severe	Gliadin, wheat	Moderate
14. 0.002	" "	"	" "	Died, 2 min.
15. 0.001	" "	"	" "	Severe
16. 0.0002	" "	Doubtful	" "	Moderate
17. 0.010	" "	Severe	" "	"
18. 0.002	" "	"	" "	Severe
19. 0.001	" "	"	" "	Moderate
20. 0.010	" "	"	" "	Died, 2 hr.
21. 0.002	" "	Moderate	" "	Severe
22. 0.001	" "	Severe	" "	"
23. 0.0002	" "	Died, 35 min.	.....	.....
Hordein, barley—				
24. 0.010	Gliadin, rye	Severe	Hordein, barley	Severe
25. 0.002	" "	"	" "	Moderate
26. 0.001	" "	Died, 10 min.	.....	.....
27. 0.0002	" "	Severe	Hordein, barley	Moderate
Gliadin, rye—				
28. 0.010	Hordein, barley	"	Gliadin, rye	Severe
29. 0.002	" "	"	.....	.....
30. 0.001	" "	"	Gliadin, rye	Moderate
31. 0.0002	" "	"	.....	.....

* The terms used to describe the outcome of the experiments and also the methods employed in conducting them are explained in our previous paper, *loc. cit.*

Thus, of 12 guinea-pigs sensitized to hordein from barley (experiments 1–12), all reacted severely when injected after three weeks with gliadin from wheat, and of these, three died. The survivors were still sensitive to hordein from barley but they reacted much less strongly than they would if they had not been injected with gliadin. Of 11 guinea-pigs sensitized to gliadin from wheat, experiments 13–23, all but one reacted strongly to hordein from barley and one died; however, these reactions were distinctly less severe than the reactions usually obtained when the second injection is gliadin from wheat, for under these conditions a large proportion of the animals

die. After the guinea-pigs recovered they were still strongly reactive to gliadin from wheat—more so than were those treated with hordein-gliadin-hordein solutions to the second dose of hordein. With gliadin from rye and hordein from barley similar inter-reactions were also observed, as shown by experiments 24–31.

From the results of these experiments it is apparent that our preparations of gliadin from wheat or rye and hordein from barley are, from the standpoint of the anaphylaxis reaction, very closely related to one another. That gliadin and hordein are not identical proteins, however, has been shown by chemical comparisons. The results of these biological reactions are in harmony with the chemical reactions, since the symptoms caused by the heterologous proteins are definitely less severe than are those caused by the homologous proteins when used in corresponding doses. The fact that after recovery from an intoxicating dose of 0.100 gm. of the heterologous protein the animal is still more or less sensitive to the homologous protein, suggests the possibility that both gliadin and hordein contain reactive groups which are not saturated by the heterologous protein; for guinea-pigs after recovering from a 0.100 gm. dose of an homologous vegetable protein are usually entirely refractory to this same protein. The presence in both hordein and wheat gliadin of common and also specific antigens is thus indicated, so that the reaction after the first injection with the heterologous protein can be ascribed to the presence of a common antigen, and the reaction after the second injection of the original protein to another specific antigen not present in the heterologous protein.

If the inter-reactions between hordein and gliadin depend upon the presence in each protein of only homologous antigens—whether these be identical proteins common to each grain, or identical antigenic radicals in one and the same protein molecule—after sensitizing guinea-pigs with hordein, and then saturating them with gliadin, they should not then be sensitive to hordein, or vice versa. If, on the other hand, the two proteins also contain specific reactive groups not common to both of them, the animal, sensitized with one, and saturated with the other, should still react when injected with the protein with which it had originally been sensitized. Evidence of common and specific antigens in hordein and gliadin

ought, therefore, to be shown by sensitizing the animal to either of these proteins, and then saturating it with the other. Under such conditions the saturated animal should still react to the specific antigen belonging to the protein used in the intoxicating dose, and, as is shown in Table 3, this is the case.

In another article[1] one of us showed that the saturation principle, as applied to the precipitin and agglutinin reactions, can also be applied to the anaphylaxis reaction for the purpose of testing the presence of multiple antigens. Thus, if an animal is sensitized to two or more proteins, it can be made refractory to one of them by injection of a sufficient quantity of that protein in one or several non-fatal doses, but is still capable of reacting to the other protein or proteins with which it was sensitized. This use of the specific refractory condition following anaphylactic reactions is based on the view of Friedberger that this refractory condition depends upon exhaustion or saturation of the anaphylactic antibodies in the sensitive animal. Our extensive experience with this phase of anaphylaxis has been in entire harmony with this hypothesis, and the recent work of Weil and Coca[2] seems to be most convincing as to its correctness. A test was made of the applicability of this saturation principle for the detection of multiple antigens and antibodies in experiments with vegetable proteins, as our previous experience had been with animal proteins. The results are shown in Table 2.

Here the guinea-pigs were sensitized with a mixture of the globulin from squash-seed together with gliadin from wheat, and after three weeks they were saturated with one of these proteins, and found to be still reactive to the other, thus establishing the reliability of the saturation method for the detection of multiple antigens. One defect of this method, however, is that animals which have reacted to one antigen will not again react so strongly to another antigen as will animals which have not already been through a series of reactions. Animals sensitized either with globulin from the squash-seed, or with gliadin from wheat, will usually react very severely, often fatally, to an injection of 0.020 to 0.100 gm.

[1] Wells, *Jour. Infect. Dis.*, 1911, 9, p. 147.

[2] Weil and Coca, *Proc. Soc. Exper. Biol. and Med.*, 1912, 9, p. 147; *Ztschr f. Immunitätsf.*, 1913, 17, p. 141.

of the homologous protein, but, as is shown in Table 2, after recovering from reactions with either one of these proteins, our animals, which had been sensitized to both of these proteins, usually developed only moderate reactions with the other protein, severe reactions being rarely obtained. Although the reduced sharpness of the reaction somewhat lowers the value of this procedure for detecting mixtures of antigens, nevertheless we have applied it with definite results to the problem of the relation of hordein and gliadin, as shown in Table 3.

TABLE 2.

SATURATION EXPERIMENTS WITH SQUASH-SEED GLOBULIN AND WHEAT GLIADIN.

Each animal had been sensitized with a mixture of 0.005 gm. squash-seed globulin and 0.005 gm. wheat gliadin three weeks before the first reinjection.

First Reinjection (21-Days After Sensitization) gm.	Result	Second Reinjection (24 Hrs. Later) gm.	Result	Third Reinjection (48 Hrs. Later) gm.	Result
Squash-seed globulin—		*Squash-seed globulin*			
1. 0.010	Moderate	0.060	Moderate	0.100 gliadin	Severe
2. 0.020	Severe	0.040	Slight	0.100 squash-seed globulin	0 (control)
3. 0.020	"	0.040	"	0.100 gliadin	Moderate
4. 0.020	"	0.060	Moderate	0.100 "	Severe
5. 0.005	.....	0.040	"	0.050 squash-seed globulin	0 (control)
6. 0.010	Slight	0.050	"	0.060 gliadin	Moderate
7. 0.010	Moderate	0.050	Doubtful	0.050 "	"
8. 0.010	"	0.050	Moderate	0.050 "	"
Gliadin—		*Gliadin*			
9. 0.005	Severe	0.050	Slight	0.100 "	0 (control)
10. 0.010	"	0.050	Moderate	0.100 squash-seed globulin	Moderate
11. 0.005	Moderate	0.050	"	0.050 gliadin	Doubtful (control)
12. 0.010	"	0.050	"	0.050 squash-seed globulin	Moderate
13. 0.010	"	0.050	Slight	0.050 " " "	"
14. 0.010	"	0.050	Doubtful	0.050 " " "	"

The results of the experiments given in Table 3 are so harmonious, that they are evidently reliable. Experiments 1–7 show conclusively that guinea-pigs sensitized with gliadin, and then saturated with gliadin by two subsequent injections of this protein, are no longer sensitive to hordein. Experiments 8–11 show equally distinctly that guinea-pigs sensitized with hordein and then saturated with hordein are rendered refractory to gliadin. The results given in Tables 1 and 3 show that gliadin from wheat and hordein from barley, although derived from plants belonging to different genera, react with one another as though they were one and the same

TABLE 3.

SATURATION EXPERIMENTS WITH GLIADIN FROM WHEAT OR RYE, AND HORDEIN FROM BARLEY.

Sensitizing Dose gm.	First Intoxicating Dose (15–21 Days Later) gm.	Result	Second Intoxicating Dose (24–48 Hrs. Later) gm.	Result	Third Intoxicating Dose (24–48 Hrs. Later) 0.100 gm.	Result
Gliadin, wheat—	*Gliadin, wheat—*		*Gliadin, wheat—*			
1. 0.010.....	0.005	Slight	0.020	Slight	Gliadin, wheat	0 (control)
2. 0.010.....	0.010	Severe	0.050	0	Hordein, barley	0
3. 0.010.....	0.005	Moderate	0.020	0	" "	0
4. 0.010.....	0.008	Severe	0.020	0	" "	0
5. 0.010.....	0.005	Slight	0.020	0	" "	0
6. 0.010.....	0.005	Moderate	0.020	0	" "	0
7. 0.010.....	0.007	Died	.....	.....	.....	.....
Hordein, barley—	*Hordein, barley—*		*Hordein, barley—*			
8. 0.010.....	0.005	Moderate	0.050	0	Gliadin, wheat	0
9. 0.010.....	0.010	Severe	0.100	0	" "	0
10. 0.010.....	0.010	"	0.100	Slight	" "	0
11. 0.010.....	0.005	Slight	0.100	0	" "	0
	Gliadin, wheat—		*Gliadin, wheat—*			
12. 0.010.....	0.020	Moderate	0.100	0	" "	0 (control)
13. 0.010.....	0.040	"	0.100	0	Hordein, barley	Severe
14. 0.010.....	0.020	"	0.100	0	" "	"
15. 0.010.....	0.020	"	0.100	0	" "	"
	Gliadin, rye—		*Gliadin, rye—*			
16. 0.010.....	0.020	Slight	0.100	Slight	" "	Moderate
17. 0.010.....	0.050	"	0.100	"	" "	Severe
18. 0.010.....	0.040	"	0.100	"	" "	Moderate
19. 0.010.....	0.050	"	0.100	"	" "	Severe
	Gliadin, wheat—		*Gliadin, wheat—*			
20. 0.010.....	0.020	Severe	0.100	0	Hordein, barley	Moderate
21. 0.010.....	0.030	"	0.100	0	" "	"
22. 0.010.....	0.040	"	0.100	0	" "	"
23. 0.010.....	0.030	"	0.100	0	" "	Died 90 min.
Gliadin, wheat—	*Hordein, barley—*		*Hordein, barley—*			
24. 0.010.....	0.050	Moderate	0.100	Slight	Gliadin, wheat	Moderate
25. 0.010.....	0.050	"	0.100	Doubtful	" "	"
26. 0.010.....	0.050	"	0.100	"	" "	"
27. 0.010.....	0.050	"	0.100	Slight	" "	"
Gliadin, rye—						
28. 0.010.....	0.100	Severe	0.100	Doubtful	Gliadin, rye	Severe
29. 0.010.....	0.100	Moderate	0.100	0	Hordein, barley	0 (control)
30. 0.010.....	0.100	"	0.100	0	Gliadin, rye	Severe
31. 0.010.....	0.100	"	0.100	0	" "	"
Hordein, barley—	*Gliadin, rye—*		*Gliadin, rye—*			
32. 0.010.....	0.100	Slight	0.100	0	Hordein, barley	"
33. 0.010.....	0.100	Severe	0.100	0	Gliadin, rye	0 (control)
34. 0.010.....	0.100	"	0.100	0	Hordein, barley	Severe
35. 0.010.....	0.100	"	0.100	0	" "	"
Gliadin, wheat—	*Hordein, barley—*		*Hordein, barley—*			
36. 0.010.....	0.050	"	0.100	Slight	Gliadin, wheat	"
37. 0.010.....	0.050	"	0.100	Doubtful	Hordein, barley	0 (control)
38. 0.010.....	0.050	"	0.100	Slight	Gliadin, wheat	Moderate
39. 0.010.....	0.060	"	0.100	"	" "	Severe

protein; in other words, gliadin and hordein appear to contain one or more *common* antigenic groups.

Experiments 1–11, however, do not show whether or not gliadin

and hordein also contain antigenic groups *specific* for each protein. Experiments 12–39 give strong evidence that specific groups are present in these proteins. Thus, when guinea-pigs were sensitized with one of these proteins, and then saturated with the other, it was found that the saturated animals were still sensitive to the protein with which they were originally sensitized. Since this result was obtained in every one of 24 experiments, any element of accident is excluded. As the animals sensitized with hordein and saturated with gliadin were still sensitive to hordein, the assumption is justified that hordein contains an antigen not present in gliadin, and conversely, gliadin contains an antigen not present in hordein. We must consequently conclude that gliadin (whether from wheat or rye) and hordein from barley contain both common and specific antigens. The reactions given in Table 3 may, therefore, be explained in the following way:

If we designate the common antigen as C, the specific antigen of gliadin as G, and the specific antigen of hordein as H, we may imagine the following situation: Gliadin will consist of GC and hordein of HC, and the reactions may be indicated by the following scheme:

First Injection	Second Injection	Result	Third Injection	Result	Explanation
(*a*) HC.....	GC	+	HC	+	1st+reaction is with antibody for C 2d+reaction is with antibody for H
(*b*) GC.....	HC	+	GC	+	1st+reaction is with antibody for C 2d+reaction is with antibody for G
(*c*) HC.....	HC	++	GC	−	1st ++reaction is with antibodies for H and C 2d−reaction, because no antibodies are present for G or C
(*d*) GC.....	GC	++	HC	−	1st++reaction is with antibodies for G and C 2d−reaction, because no antibodies are present for H or C

(1) An animal (*a*) sensitized to HC reacts to GC because of the presence of C in each protein, but the reaction is less severe than with HC, presumably because only one radical (C) is reacting.

(2) An animal (*c*) sensitized with HC and saturated with HC will not react with GC, because the antibodies for C are saturated. Similarly, if sensitized (*d*) with GC and saturated with GC it will

no longer react to HC, because of the exhaustion of the antibody for C.

(3) But if sensitized (*a*) with HC and saturated with GC it will still be sensitive to HC, since the antibodies for H remain, but the reaction will be less severe than if HC was injected for the first intoxicating dose since then both antigens react. Similarly (*b*) for the GC+HC+GC series.

Since the above conditions are exactly fulfilled in the experiments in this series, the conclusion seems warranted that *hordein and gliadin each contain distinct specific antigens as well as common antigens*. We must conclude from the results of our experiments, either that our preparations of gliadin and hordein each contain two different *proteins*, one of which is common to both preparations, or that they contain at least two reactive *groups*, one of which is common to both proteins, each of which groups behaves as a distinct antigen when injected into guinea-pigs. The chemical evidence supports the latter interpretation. We thus find that the group reactions which characterize species of close biological relationship, whether bacteria, plants, or animals, are also exhibited by purified proteins of similar chemical nature isolated from related species.

GLUTENIN EXPERIMENTS.

Experiments with glutenin gave the results shown in Table 4.

Animals sensitized with glutenin reacted severely to wheat gliadin and were then almost entirely refractory to glutenin. They did *not* react to hordein, and were *not* made refractory to glutenin by hordein. If sensitized with glutenin and then saturated with glutenin they were nearly, but not quite refractory to gliadin.

If sensitized with gliadin they reacted if anything, stronger to glutenin than if sensitized with glutenin, but were rendered little if at all refractory to gliadin by saturation with glutenin. When sensitized with hordein they did not react with glutenin, and were made only partly refractory to hordein.

Since glutenin and gliadin are the chief constituents of wheat gluten, and must be separated from one another to obtain the preparation used for this work, it might be assumed that the reactions here reported were caused by an incomplete separation of these

TABLE 4.

REACTIONS WITH GLUTENIN FROM WHEAT.

Sensitizing Dose gm.	Second Dose (15–21 Days Later)	Result	Third Dose (24–48 Hrs. Later)	Result	Fourth Dose (24–48 Hrs. Later)	Result
Glutenin, wheat:						
1. 0.002...	Glutenin, wheat	Severe	Gliadin, wheat	0	.....	.....
2. 0.010...	" "	Moderate	Glutenin, wheat	0	Gliadin, wheat	Slight
3. 0.010...	" "	Slight	" "	0	" "	"
4. 0.010...	" "	0	" "	0	" "	0
5. 0.002...	" "	0	" "	0	" "	0
6. 0.010...	" "	Moderate	.....	.....	.....	.....
7. 0.010...	" "	Slight	.....	.....	.....	.....
8. 0.010...	" "	Doubtful	Glutenin, wheat	0	Gliadin, wheat	0
9. 0.002...	" "	0	" "	0	" "	0
10. 0.002 ..	" "	Slight	" "	0	" "	0
11. 0.002...	" "	Moderate	" "	0	" "	0
12. 0.010...	Gliadin, wheat	Severe	Glutenin, wheat	Slight	.....	.....
13. 0.002...	" "	"	Gliadin, wheat	0	.....	.....
14. 0.001...	" "	Died 75 min.	.....	.....	.....	.....
15. 0.0002..	" "	Severe	Glutenin, wheat	Doubtful	.....	.....
16. 0.010...	" "	"	Gliadin, wheat	0	Glutenin, wheat	0
17. 0.010...	" "	"	" "	0	" "	Doubtful
18. 0.010...	" "	"	Glutenin, wheat	Slight	.....	.....
19. 0.010...	" "	"	" "	Moderate	.....	.....
20. 0.010...	Gliadin, rye	0	" "	Doubtful	.....	.....
21. 0.002...	" "	0	" "	Slight	.....	.....
22. 0.001...	" "	Moderate	" "	Moderate	.....	.....
23. 0.0002..	" "	Slight	" "	"	.....	.....
24. 0.001...	" "	0	" "	0	.....	.....
25. 0.001...	" "	0	" "	0	.....	.....
26. 0.010...	" "	Slight	Gliadin, rye	0	Glutenin, wheat	Slight
27. 0.002...	" "	"	" "	0	" "	"
28. 0.001...	" "	"	" "	0	" "	"
29. 0.0002..	" "	Died 30 min.	.....	.....	.....	.....
Gliadin, wheat:						
30. 0.010...	Glutenin, wheat	Severe	Gliadin, wheat	Moderate	.....	.....
31. 0.002...	" "	Moderate	.....	.....	.....	.....
32. 0.0002..	" "	Severe	.....	.....	.....	.....
33. 0.010...	" "	"	Gliadin, wheat	Moderate	.....	.....
34. 0.002...	" "	Died 2 hrs.	.....	.....	.....	.....
35. 0.001...	" "	Severe	Glutenin, wheat	0	.....	.....
36. 0.0002..	" "	"	Gliadin, wheat	Moderate	.....	.....
37. 0.010...	" "	Moderate	Glutenin, wheat	0	Gliadin, wheat	Slight
38. 0.010...	" "	"	" "	0	" "	"
39. 0.010...	" "	Severe	" "	0	" "	Moderate
40. 0.020...	" "	"	" "	0	" "	Severe
41. 0.010...	" "	Moderate	" "	0	" "	Moderate
42. 0.020...	" "	"	" "	0	" "	Severe
43. 0.010...	" "	Severe	" "	0	" "	Moderate
44. 0.002...	" "	"	" "	0	" "	"
45. 0.002...	" "	Moderate	" "	0	" "	"
Gliadin, rye:						
46. 0.010...	" "	"	" "	Slight	Gliadin, rye	Severe
47. 0.002...	" "	Slight	" "	Doubtful	.....	.....
48. 0.001...	" "	"	" "	0	Gliadin, rye	Severe
49. 0.0002..	" "	Moderate	" "	0	.....	.....
50. 0.010...	" "	"	" "	0	Gliadin, rye	Slight
51. 0.002...	" "	Severe	" "	0	" "	"
52. 0.001...	" "	"	" "	0	" "	"
53. 0.0002..	" "	Moderate	" "	0	" "	"
Hordein, barley:						
54. 0.010...	" "	0	Hordein, barley	Slight	.....	.....
55. 0.010...	" "	0	" "	Moderate	.....	.....
56. 0.010...	" "	0	" "	"	.....	.....
57. 0.010...	" "	0	" "	Severe	.....	.....
58. 0.010...	" "	Doubtful	" "	0	Hordein, barley	Slight
59. 0.002...	" "	0	" "	0	" "	"
60. 0.001...	" "	0	" "	0	" "	"
61. 0.0002..	" "	0	" "	0	" "	"

TABLE 4—*Continued.*

Sensitizing Dose gm.	Second Dose (15–21 Days Later)	Result	Third Dose (24–48 Hrs. Later)	Result	Fourth Dose (24–48 Hrs. Later)	Result
Glutenin, wheat:						
62. 0.010...	Hordein, barley	0	Glutenin, wheat	Slight	.....	.....
63. 0.002...	" "	0	" "	"	.....	.....
64. 0.001...	" "	0	" "	"	.....	.....
65. 0.0002..	" "	0	" "	"	.....	.....
66. 0.010...	" "	0	" "	"	.....	.....
67. 0.010...	" "	0	" "	"	.....	.....
68. 0.010...	" "	0	" "	"	.....	.....
69. 0.010...	" "	0	" "	"	.....	.....
70. 0.005...	" "	0	Hordein, barley	0	Glutenin, wheat	Slight
71. 0.001...	" "	0	" "	0	" "	Doubtful
Mixture of glutenin and gliadin, wheat:						
72. 0.010...	" "	Moderate	Glutenin, wheat	Slight	Gliadin, wheat	Severe
73. 0.002...	" "	"	" "	"	" "	Moderate
74. 0.001...	" "	Severe	" "	Moderate	" "	"
75. 0.0002..	" "	"	" "	"	" "	Severe

SUMMARY.

Glutenin vs. glutenin: 11 experiments, severe 1, moderate 3, slight 3, doubtful 1, no symptoms 3.
Glutenin vs. wheat gliadin: 8 experiments, severe 7, fatal 1.
Glutenin vs. rye gliadin: 10 experiments, moderate 1, slight 4, fatal 1, no symptoms 4.
Glutenin vs. hordein: 10 experiments, no symptoms in any.
Wheat gliadin vs. glutenin: 16 experiments, fatal 1, severe 9, moderate 6.
Rye gliadin vs. glutenin: 8 experiments, severe 2, moderate 4, slight 2.
Hordein vs. glutenin: 8 experiments, doubtful 1, none 7, no symptoms 5.
Sensitized with glutenin and saturated with glutenin: protects vs. wheat gliadin.
Sensitized with glutenin and saturated with hordein: not protected vs. glutenin.
Sensitized with gliadin and saturated with glutenin: only slight protection.
Sensitized with glutenin+wheat gliadin react well with hordein, also with glutenin and wheat gliadin.

proteins. We have already given our reasons for believing that the separation was nearly complete, and that no more than traces of gliadin were present in our preparation of glutenin, or of glutenin in the preparation of gliadin. If this assumption is correct, our experiments justify the conclusion that gliadin and glutenin contain common reacting groups, for those animals sensitized by the smallest quantity of either one of these proteins reacted quite as severely, when intoxicated with the heterologous protein, as did those intoxicated with the homologous protein. In other words, gliadin and glutenin react with one another almost as if they were identical proteins, although all of the chemical evidence, especially that relating to their amino-acid make-up, shows them to be distinctly different proteins. The fact that hordein, which readily reacts with gliadin, fails to react with glutenin also supports this view, for, if the glutenin preparations contained sufficient gliadin

to render the animals sensitive to gliadin we should certainly expect them to show some symptoms at least when subsequently treated with hordein.

These experiments give no clue as to whether or not the gliadin preparation is contaminated with glutenin, and at present there appears to be no means whereby this question can be settled beyond a doubt. The commonly accepted opinion that gliadin yields no lysine on hydrolysis, whereas glutenin does, might be offered as evidence on this point. One of us, however, has recently found[1] that a supposedly pure preparation of gliadin, which, when tested according to Kossel's method, gave no indication of yielding any lysine, did, in fact, yield a very small amount, which could be detected when changes in Kossel's method were made with a veiw to isolating very small quantities of the picrate of this amino-acid. Investigations now in progress have given, as yet, no evidence indicating the presence of glutenin in the preparation of gliadin under examination.

LOCAL PERITONEAL REACTIONS.

In the course of our experiments, involving repeated injections of foreign proteins into the same animal, we have noticed a somewhat inconstant, but often striking phenomenon, to which we wish to call attention. When an animal which has been given the large second or intoxicating dose of the vegetable protein into the peritoneum, is given 24 to 72 hours later another intraperitoneal injection of the same protein, to which it is now, as a rule, entirely refractory as regards anaphylactic reaction, it may exhibit symptoms of a severe, but very transient peritoneal irritation, although the previous dose of the same protein had had no similar effect. About 15 to 60 seconds after injection the animal dashes about madly for a few seconds, jumps up and down, arches the back as if trying to relieve intraperitoneal pressure, and seems in great distress for one-half to two minutes; the symptoms cease quickly, and after one or two minutes more the animal seems entirely well. Apparently the reaction is, at least partly, specific, for unless the two injections are with the same protein the effect is not observed.

[1] Osborne and Mendel, *Jour. Biol. Chem.*, 1912, 12, p. 473.

Since first observing this phenomenon we have made note of its occurrence, and find that it is usually, but not always, exhibited, and in one set of experiments it may be marked and yet be entirely missing in a duplicate set made at another time, and that there are many other variations and discrepancies, so that we cannot as yet interpret it. Possibly it is in the nature of a local sensitization and reaction.

SUMMARY.

1. The preparations of proteins used for the experiments described were very carefully purified in order to separate them as completely as possible from all other proteins.

2. These proteins were hordein from barley, glutenin from wheat, and gliadin from both wheat and rye. Chemical investigations have established such marked differences between hordein, glutenin, and gliadin that they are commonly regarded as well-established individual proteins. Between gliadin from wheat and gliadin from rye, no difference has been observed sufficient to justify the assumption that these are different proteins.

3. Guinea-pigs sensitized with gliadin from wheat, or rye, give strong anaphylactic reactions with hordein from barley, but these are not as strong as the reactions obtained with the homologous protein. Similar results are obtained if the sensitizing protein is hordein and the second injection is gliadin. We here have a common anaphylaxis reaction developed by two chemically distinct, but similar, proteins of different biological origin, thus indicating that the specificity of this reaction is determined by the chemical constitution of the protein rather than by its biological origin. This is in harmony with the fact that chemically closely related proteins have, as yet, been found only in tissues that are biologically nearly related.

4. Complete protection to subsequent injection of the homologous protein was not afforded by a reaction to the heterologous protein, thus indicating the presence of two or more individual proteins in the preparations of gliadin and hordein, one of which is common to both, or else the presence in gliadin and hordein of both common and specific reactive groups. The chemical evidence is in favor of the latter conclusion.

5. The foregoing indications are supported by saturation experiments, which show that when guinea-pigs are sensitized with either gliadin or hordein, and then saturated with the heterologous protein, they still react strongly when injected with the homologous protein.

6. Gliadin and glutenin react anaphylactically with one another, although chemical comparisons have shown them to be proteins of distinctly different types. Evidence was obtained that the reactions between these proteins should not be ascribed to contamination of the preparations with one another, i.e., to an incomplete separation of the two. The conclusion appears justified that these chemically distinct proteins contain common reactive groups.

7. Guinea-pigs sensitized with glutenin do not react anaphylactically with hordein, thus showing that the reaction between gliadin and glutenin is not caused by an incomplete separation of these latter proteins, but by reactive groups common to gliadin and glutenin, but absent from hordein.

8. From the results of these experiments it seems probable that the entire protein molecule is not involved in the specific character of the anaphylaxis reaction, but this is developed by certain groups contained therein, and that one and the same protein molecule may contain two or more such groups. It may well be that the intact protein molecule is involved in the reaction (for there is but little evidence that anything less than an intact protein molecule is capable of producing the typical reaction), but that certain groups determine the specificity. Such a conclusion cannot be accepted as final until we have some means whereby the chemical individuality of a protein can be established. Until then the possibility will remain that our so-called pure preparations of proteins consist of mixtures, or combinations, of proteins which have thus far resisted all efforts to separate them. In this latter case the reactions here attributed to groups in one protein molecule might be caused by individual proteins contained in the preparations made by the methods now in use.

9. These experiments demonstrate that the "group reactions," characteristic of biological reactions between closely related species,

which usually have been interpreted as indicating the presence in related organisms of identical as well as distinct proteins, can really be exhibited by single isolated proteins from related organisms. In other words, biological relationship and chemical relationship seem to be much the same.

10. Attention is also called to certain other observations: (*a*) that animals sensitized with two proteins will, as is well known, react with either, and that after recovery from reaction with one protein the reaction given with the second protein is less severe than it would be if the animal had not already passed through an anaphylactic intoxication; (*b*) that after injection with an intoxicating dose of a vegetable protein, another injection with the same protein 24 to 72 hours later, when the animal is usually insusceptible, so far as constitutional symptoms are concerned, often produces a severe, transient peritoneal irritation, which seems to be in the nature of a specific local reaction.